



Annual Reports :: Year 6 :: Carnegie Institution of Washington

Project Report: Astrobiotechnology

<b>Project Investigators:</b>	<b>Robert Hazen , Wesley Huntress , Timothy McCoy , James Scott , Andrew Steele , Ed Vicenzi</b>
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## Project Progress

The development of new technology to carry out *in situ* experiments to address astrobiological questions is an important goal of NAI, underscored this year by the formation of an Astrobiotechnology Focus Group. An early action by the Focus Group was to secure funding to hold a workshop at the Carnegie Institution of Washington (CIW) this fall (8–10 September 2004). Further activities of this focus group are provided in a separate report to NAI. Within the CIW team, efforts in astrobiotechnology have focused on several aspects of life detection instrumentation for remote missions to Mars and other solar system bodies.

### 1. Development of Flight Instrumentation

With support from the NASA Astrobiology Technology for Instrument Development (ASTID) program, Co-I Steele and his collaborators have initiated work on a prototype instrument to detect prebiotic molecules and biosignatures, the Modular Assays for Solar System Exploration (MASSE). In this past year prototyping was completed for microfluidic chips to lyse gram-negative, gram-positive, and archaeal cells (Figure 1), perform clean-up and concentration of targeted biomarkers, and inoculate these onto a protein microarray. This work is being undertaken at Marshall Space Flight Center (MSFC) by the Lab-on-a-chip Development (LOCAD) group. A prototype sample preparation system has been built and is currently being tested at CIW. Optical systems to interrogate the inoculated array have been prototyped and built and tested at Montana State University, and advanced concept designs have been conceived and are currently being fabricated. A final flight design is currently being developed by Oceaneering Space Systems of Houston.

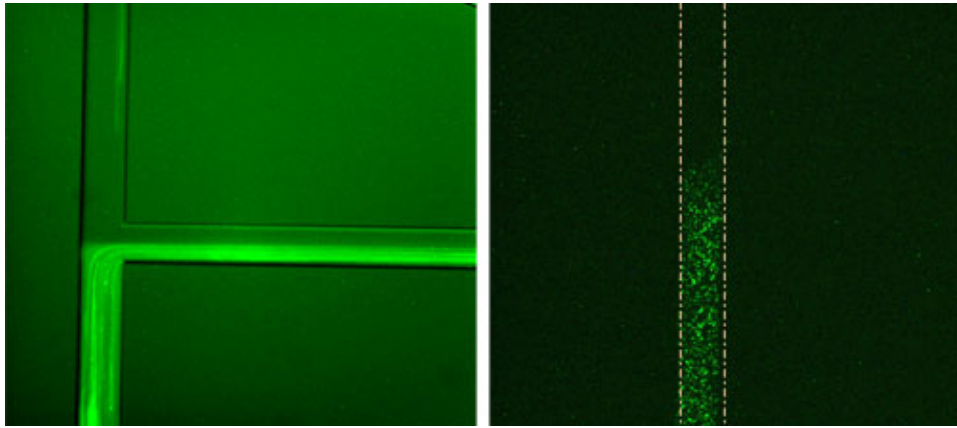


Figure 1. (a) Brightfield and fluorescence imaging combined to illustrate the NS145 chip T-junction and pathway of SYTO-9 stained *E. coli*. (b) Mixing of *H. marismortui* with the lysing agent Solution B causes complete lysis of the archaeal cell wall and a decrease in fluorescence.

The definition of protein microarray chips that contain antibodies to extinct and extant life biomarkers continued during the past year. The ability of an antibody microarray to search for hundreds of different biological molecules simultaneously, using only a few microliters of sample (all within an area of a few square millimeters on a glass slide), renders it an appealing method for *in situ* detection of “biomarkers” on the Martian surface. Largely used to investigate patterns of gene expression, this method has yet to be fully developed for microbial detection in geological samples. To test the suitability of antibody microarrays for such a purpose, we designed a microarray to detect two common bacterial antigens, lipopolysaccharide (LPS) and groEL (chaperonin 60). To evaluate possible effects of a geological sample upon the efficiency of antibody binding (e.g., changes in pH or high  $\text{Fe}_3^+$ ), we spiked simulant Martian regolith with LPS, added directly to the microarray surface without filtering, showing that LPS-specific detection was not inhibited. A more complex microarray was then developed to detect eleven biomarkers (LPS, DNA, peptidoglycan, collagen, groEL, & beta;-galactosidase, mycoplasma, K-99 pilus protein from *Escherichia coli*, free fatty acids, and nitrate reductase) in a single sample of simulant Martian regolith spiked with lyophilized *E. coli*. Further experiments including long-term monitoring of antibody stability over the time it takes to travel to Mars was instigated using a variety of active surfaces. Testing of antibodies to small molecules and fossil antigens continued, including antihopane and sterane antibodies and collagens within fossilized bones.

The group has also been testing rock crushers designed for flight onboard the Mars Science Laboratory mission. Testing for mineral carry-over from sample to sample has been completed, and testing for bacterial carry-over is being carried out by spiking basalt samples with *E. coli* and measuring the amount of cells left on the crusher after operation. The team is using real-time polymerase chain reaction (PCR), Limulus amoebocyte lyase (LAL) test (supplied by N. Wainwright of the Marine Biological Laboratory), and adenosine triphosphate (ATP) assay. Results suggest that a small amount of carry-over occurs, but further testing is underway.

## *2. Laser-Induced Breakdown Spectroscopy (LIBS)*

As a collaborative effort with Gregory Bearman of the Jet Propulsion Laboratory, Co-I Steele, Postdoctoral Fellow Fries, and others have investigated the use of laser-induced breakdown spectroscopy (LIBS) for astrobiology projects. They assembled a small LIBS device using a 337-nm N<sub>2</sub> laser. LIBS utilizes a focused laser pulse to ablate a small (~100-μm diameter) spot from a sample surface and excite that material briefly into a plasma. Since that plasma is effectively a small sample of the bulk material that is converted to a hot but rapidly cooling gas, the light emitted is effectively an atomic emission spectrum from the bulk material. This technique has been used to examine a sample of hot springs carbonate from a Mars analog location in Svalbard. The carbonate rocks are host to a photosynthesizing cryptoendolithic microbial community, which lives inside the rock at a depth of about a millimeter where it is protected from dessicating winds and ultraviolet radiation. LIBS was employed on this sample using both cryptoendolith-containing and heat-sterilized samples to test whether LIBS is useful to measure directly the presence and/or abundance of the organisms. It was determined that, for the small ultraviolet system used here, LIBS was unable to detect conclusively the presence of the organisms. However, LIBS was able to distinguish clearly the presence of even very small carbonates against basaltic groundmass. Since LIBS can be made into a very small system with no logistical requirements other than a relatively small amount of electrical power, it will likely be very useful as a triaging technique. In this role it will complement the MASSE project nicely. Relative to LIBS, MASSE will require a substantial time per sample and will deplete its store of expendable reagents with each test. LIBS can therefore be used to search for sampling locations for MASSE.

## *3. Fieldwork at Mars Analog Sites*

The Arctic Mars Analog Svalbard Expedition (AMASE) involved the participation of 18 scientists on an ice breaker for two weeks to visit the world's northernmost terrestrial hot springs and the site of the only analog to the carbonate globules found in ALH84001. This site (79° 23.294'N, 13° 26.392'E) is near the Sverrefjell volcano. Co-I Steele joined a multidisciplinary group including Tori Hoehler (of the NAI team at NASA Ames Research Center) to field test protocols and instruments for characterizing the presence of microbes in both the hot-springs and the volcanic basalts. These techniques included polymerase chain reaction (PCR) and hand-held instruments to conduct the Limulus amebocyte lyase (LAL) test (supplied by N. Wainwright, Marine Biological Laboratory) and adenosine triphosphate (ATP) assay, as well as a hand-held digital microscope. The successful field testing enabled field PCR to be conducted for the first time in the Arctic to confirm the presence of functional genes for bacterial sulphur metabolism in the Trollolsen hot springs. ATP luminometry was conducted on relic carbonate hot springs that showed the presence of cryptoendolithic communities (Figure 2). Samples returned to the laboratory have been analyzed by many techniques in order to prepare the Svalbard area to serve as a Mars analog for field testing of instrumentation for the Mars Science Lander (MSL). A second expedition will be mounted this year.

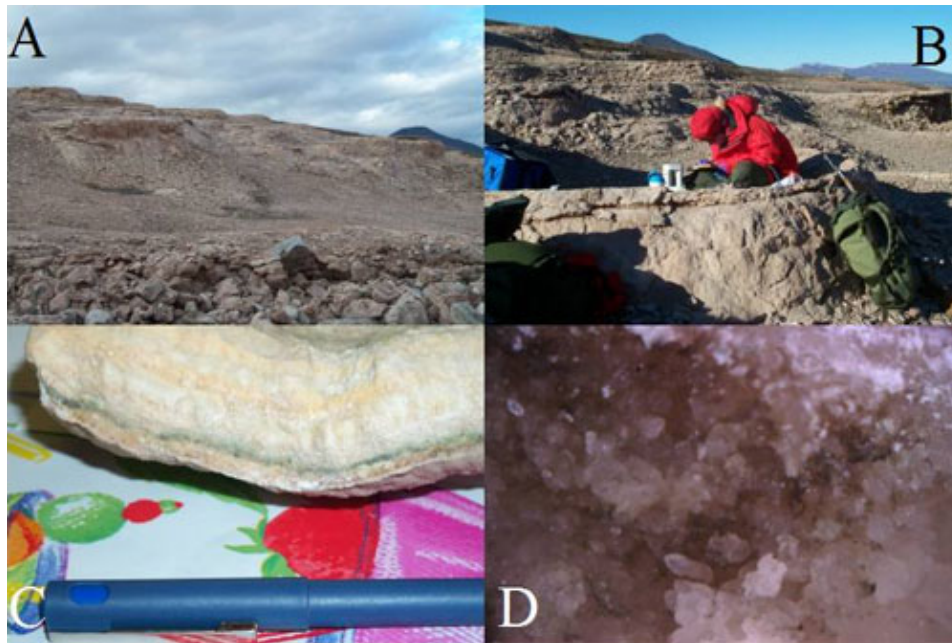


Figure 2. (A) An image of relic terraces at the AMASE site in Svalbard. (B) Picture of the ATP analysis being undertaken on the lower terrace by undergraduate M. Schweizer. (C) Image of a sample of the terrace showing the layering the carbonate and the presence of a layer of cyanobacteria typical of a cryptoendolithic community. (D) x30 magnification digital image showing the presence of dark green cyanobacterial colonies through the matrix of the sample shown in (C).

#### 4. Application of Astrobiotechnology for Origin of Life Issues

Co-Is Steele and Hazen and Postdoctoral Fellow Jake Maule applied biotechnology to improve the protocol for testing crystal surfaces for chiral selectivity. Using the unique capability of the Telechem Spotbot microarrayer to print on uneven surfaces, the group printed small volumes (0.7 nL) of fluorescently L- and D-amino acids onto feldspar, calcite, and mica surfaces, at up to 20 different dilutions, each in quintuplicate to allow for surface variation. The surfaces were then scanned for fluorescence with a Genepix 4000B scanner, washed with saline solution, and then scanned again to observe any preferential binding post-wash. In addition, “left” and “right” faces of quartz were printed with fluorescently labeled L-lysine and fluorescence quantified pre- and post-wash (Figure 3). There appears a significant and quantifiable retention of L-lysine on the right face of the crystal.

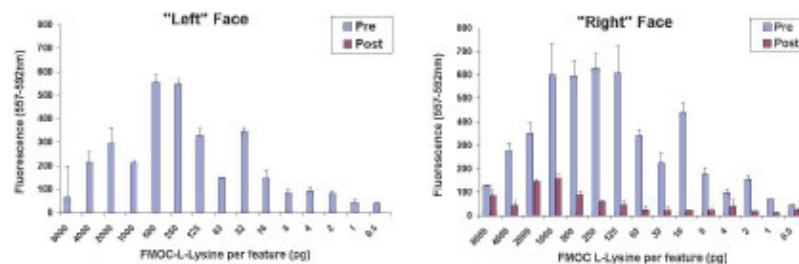


Figure 3. Preferential binding of fluorescently labeled amino acids on the left and right faces of quartz.

## Highlights

- Life–detection instrumentation has been tested in a Mars analog environment on Svalbard. This environment will be used to test flight instrumentation proposed for Mars lander missions.
- Fossil collagens in 40,000–year–old bone samples have been characterized using the world’s first antibody microarray to fossil compounds.
- High throughput has been achieved for the simultaneous screening of thousands of fluorescently labeled D and L amino acids for assessing their binding characteristics to mineral surfaces.
- Quantitative microfluidic cell lysis has been demonstrated for gram–negative, gram–positive, and archaeal cells.

## Roadmap Objectives

- **Objective No. 2.1:** Mars exploration
- **Objective No. 3.1:** Sources of prebiotic materials and catalysts
- **Objective No. 3.2:** Origins and evolution of functional biomolecules
- **Objective No. 4.2:** Foundations of complex life
- **Objective No. 7.1:** Biosignatures to be sought in Solar System materials

## Mission Involvement

<b><i>Mission Class*</i></b>	<b><i>Mission Name (for class 1 or 2) OR Concept (for class 3)</i></b>	<b><i>Type of Involvement**</i></b>
2	MSL	Planning Support
2	MSL	Instrument/Payload Development
2	ExoMars	Planning Support
2	ExoMars	Instrument/Payload Development

\* Mission Class: Select 1 of 3 Mission Class types below to classify your project:

1. Now flying OR Funded & in development (e.g., Mars Odyssey, MER 2003, Kepler)
2. Named mission under study / in development, but not yet funded (e.g., TPF, Mars Lander 2009)
3. Long–lead future mission / societal issues (e.g., far–future Mars or Europa, biomarkers, life definition)

\*\* Type of Involvement = Role / Relationship with Mission

Specify one (or more) of the following: PI, Co–I, Science Team member, planning support, data analysis, background research, instrument/payload development, research or analysis techniques, other (specify).

Co-I Steele is actively advising the Mars Exploration Program. He serves on the Organic Contamination Science Steering Group, the Mars Icy Science Team, and the MEPAG Goals Committee. Steele chairs and Postdoctoral Fellow Toporski serves on the Astrobiology Field Laboratory (AFL) Science Steering Group, a committee commissioned by MEPAG and the NASA Mars Office to define the science objectives and mission architecture for a proposed follow-on mission to MSL in 2013.

Several team members are involved in proposed experiments on MSL. Co-I McCoy is involved in a proposal for a gamma-ray spectrometer to search for H – either as ice or bound in hydrated minerals – and other elements that are concentrated by biogenic processes or a necessary agent for such processes (e.g., P, Mn, Ca). Co-Is Huntress, Scott, and Steele are involved in a proposal for a Surface Analysis for Mars (SAM) instrument. Steele is also a participant on two other proposed instruments. One is the Mars Ultraviolet Science Experiment (MUSE), designed to triage samples using deep-UV fluorescence spectroscopy to detect organic and mineral signatures at a high level of sensitivity. The other is the Rocklab experiment package, which combines a thermal evolved gas analyzer, X-ray diffraction, and high-resolution microscope to examine samples for carbon content and isotope signature, mineralogy, and imaging.

Steele and Toporski are advising ESA on the Pasteur exobiology payload and rover for the ExoMars mission. They are also collaborating on a proposal for an instrument, Molecular Biology Instrument for Life Detection (MoBILD).

## Field Expeditions

### **Field Trip Name:** Arctic Mars Analogue Svalbard Expedition (AMASE)

<b>Start Date:</b> 10 August 2003	<b>End Date:</b> 22 August 2003
<b>Continent:</b> Arctic	<b>Country:</b> Svalbard
<b>State/Province:</b>	<b>Nearest City/Town:</b> Longyearbyen
<b>Latitude:</b> 79° 23.294'N	<b>Longitude:</b> 13° 26.392'E
<b>Name of site(cave, mine, e.g.):</b> Troll Springs Sverrefjell	<b>Keywords:</b>

**Description of Work:** Field testing of protocols and instruments for characterizing the presence of microbes in hydrothermal and volcanic analogs to sites on Mars.

### **Members Involved:**

## Cross Team Collaborations

Co-I Steele maintains collaborations with N. Wainwright (MBL), Tori Hoehler (ARC), and V. Parro Garcia (CAB).